AMENDMENTS TO THE SPECIFICATION

Page 2, before line 1, insert the following replacement paragraph:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a division of U.S. patent application Serial No. 09/218,176 filed December 22, 1998, now U.S. Patent 6,171,584, which in turn is a ~~Continuation Application~~ continuation of ~~Parent Application~~ U.S. patent application Serial No. 08/679,048 filed July 12, 1996, which is a continuation-in-part of U.S. patent application Serial No. 08/482,577, filed July 6, 1995. The ~~disclosure~~ disclosures of the prior ~~application(s) is~~ applications are ~~hereby~~ incorporated by reference herein in ~~its~~ their entirety.

D1

Page 5, third full paragraph, insert the following replacement paragraph:

SEQ ID ~~NO. 5~~ NOS:5 and 6 show ~~shows~~ the nucleotide sequence of the human MP121 gene at the exon/intron junctions. ~~The nucleotides from both exons are marked by capital letters those of the intron by small letters.~~

D2

Page 5, fourth full paragraph, onto page 6, insert the following replacement paragraph:

Figure 1 shows a comparison of the amino acid sequence of human MP121 (SEQ ID NO:7) with some members of the TGF-β family (inhibin α and β chains) (SEQ ID NOS:8-10) starting at the first of the seven conserved cysteine residues. * denotes that the amino acid is the same in all compared proteins; + denotes that the amino acid corresponds in at least one of the proteins compared to humna MP121.

D3

Page 6, first full paragraph, insert the following replacement paragraph:

Figure 2 shows the nucleotide sequences (SEQ ID NOS:11-32) of the oligonucleotide primers which were used in the present invention and a comparison of these sequences with known members of the TGF-β family. M denotes A or C, S denotes C or G, R denotes A or G and K denotes G or

D4

D4 T. Figure 2a shows the sequence of primer OD~~,~~ (SEQ ID NO:11). Figure 2b shows the sequence of primer OID (SEQ ID NO:22).

Page 6, third full paragraph, insert the following replacement paragraph:

Figure 4 shows the expression of MP121 compared to activin β_A and β_B in various mouse tissues and is an autoradiogram after gel analysis of an RNase protection assay using specific probes against activin ~~βA (BA)~~ β_A (β_A), activin ~~βB (βB)~~ β_B (β_B), MP121 and against GAPDH for the control. Total RNA was tested which ~~has~~ had been isolated from various mouse tissues (Lane 1: brain, Lane 2: heart, Lane 3: kidney, Lane 4: liver, Lane 5: lung, Lane 6: muscle, Lane 9: ovary, Lane 10: spleen, Lane 11: testes) from embryonic stem cells (Lane 12: CJ7) and from yeast (Lane 13) as a control. No RNA was used in Lane 14 as a control. The unprotected antisense RNA probes used for the hybridization are applied in ~~lanes~~ Lanes 8 and 15 and the expected fragment size is indicated in brackets in the right margin. The bands of the protected fragments are labeled in the left margin. PBR322 restricted with ~~Map~~ Msp I (Biolabs #303) and end-labeled with ~~γ-32p-ATP~~ γ-^{32}P-ATP (Amersham) was used as the marker (Lane 7).

D5

Page 6, sixth full paragraph, insert the following replacement paragraph:

Figure 7 shows the stimulation of nerve fibre outgrowth from the embryonic retina by treatment with partially purified ~~PM121~~ MP121. Dark-field microscopy of living cultures shows nerve fibre outgrowth from explanted chicken retina after 4 days in culture in the presence of 5 ng/ml of partially purified MP121.

D6

Page 21, line 20 to page 22, line 9, insert the following replacement paragraph:

The clone was extended to the 3' end of the cDNA according to the method described in detail by Frohmann (published by Perkin-Elmer Corp., Amplifications, 5, 11-15 (1990)). The same liver mRNA which had been used to isolate the first MP121 fragment was reversely transcribed as described above using oligo dT (16mer) linked to the adapter primer (AGAATTCGCATGCCATGGTCGACGAAGC-T_{16}) (SEQ ID NO:33). The amplification was

D7

carried out using the adapter primer (AGAATTCGCATGCCATGGTCGACG) (SEQ ID NO:34) and an internal primer (GGCTACGCCATGAACTTCTGCATA) (SEQ ID NO:35) prepared from the MP121 sequence. The amplification products were prepared using a further internal primer (ACATAGCAGGCATGCCTGGTATTG) (SEQ ID NO:36) prepared from the MP121 sequence and with the adapter primer. After restriction with Sph I the reamplification products were cloned into the vector pT7/T3 U19 (Pharmacia) which had been cleaved in the same way and sequenced. The clones were characterized by their sequence overlap with the already known part of the MP121 sequence. One clone, named p121Lt 3' MP13, was used to isolate a Nco I (made blunt using T4 polymerase)/Sph I fragment. This fragment was cloned into one of the above-mentioned pSK-MP121 (OD/OID) vectors whose OD primer sequence was orientated towards the T7 primer of the pSK multiple cloning site. For this the vector was cleaved with Sph I and Sma I. The construct was named pMP121DFus6. It comprises the MP121 sequence from position 922 to 1360 as shown in SEQ ID ~~NO:1~~ NO:1.

D7

Page 22, lines 10-22, insert the following replacement paragraph:

1.9 A Dde I fragment of pMP121DFus6, which extends from position 931 to 1304 in SEQ ID ~~NO:1~~ NO:1, was used to screen a human liver cDNA library (Clontech, #HL3006b, lot 36223) as described in detail by Ausubel et al., (Current Protocols in Molecular Biology published by Greene Publishing Associates and Wiley-Interscience (1989)). Twenty-four mixed plaques were picked from 8.1 x 10^5 phages and separated. From this, 10 clones which yielded a positive signal using primer LO2 (ACATAGCAGGCATGCCTGGTATTG) (SEQ ID NO:36) and LOI1 (CTGCAGCTGTGTTGGCCTTGAGA) (SEQ ID NO:37) from the Dde I fragment were selected and separated. The cDNA was isolated from the phages by means of an EcoRI restriction and cloned into the pBluescript SK vector which had also been cleaved with EcoRI.

D8

Page 23, lines 1-20, insert the following replacement paragraph:

1.10 Isolation of the MP121 cDNA and genomic DNA from the mouse: The sequence information from the human MP121 sequence was used to isolate the MP121 sequence from the mouse. The methods used for this are all known to a person skilled in the art and are described, for example, in Current Protocols in Molecular Biology (Ausubel et al., Greene Publishing Associates and Wiley-Interscience, Wiley & Sons, 1987-1995) or in Molecular Cloning (Sambrook et al., second edition, Cold Spring Harbour Laboratory Press, 1989). Firstly, the primers ACGAATTCCGACGAGGCATCGACTGC (SEQ ID NO:38) and GCGTCGACTACCATGTCAGGTATGTC (SEQ ID NO:39) derived from the human MP121 sequence containing additional restriction cleavage sites at the 5' end (EcoRI or SalI) were synthesized. These primers were used for amplification on genomic mouse DNA. The 0.35 kb fragment which results was subcloned in the ~~Bluescipt~~ Bluescript vector (Stratagene) and used as a radioactive probe. A λ bank with genomic mouse DNA as well as a bank with cDNA was screened according to standard methods. The cDNA was synthesized from RNA, which had been isolated from mouse liver and cloned into λgt10 using EoRI/NotI linkers.

D9

Page 23, lines 21-34, insert the following replacement paragraph:

MP121 clones were isolated from the genomic as well as from the cDNA bank. A cDNA containing the complete coding sequence was subcloned into the EcoRI cleavage site of the Bluescript vector SK (Stratagene) and the resulting plasmid SKMP121 mouse was deposited on ~~the~~ 10.05.1995 at the DSM (DSM 9964). Complete sequencing resulted in the sequence shown in SEQ ID ~~NO.3~~ NO:3. The start codon begins at position 131 in SEQ ID ~~NO.3~~ NO:3 and ends at the stop codon starting at position 1187. The protein derived from the sequence is shown in SEQ ID ~~NO.4~~ NO:4. Subcloning and analyzing clones containing MP121 from the genomic bank showed that the MP121 sequence contains an intron in the propeptide part of ca. 5.5 kb. This intron is located between positions 446 and 447 in SEQ ID ~~NO.3~~ NO:3. The exon/intron junctions are shown in SEQ ID ~~NO.5~~ NOS:5 and 6.

D10

Page 24, line 12 to page 25, line 30, insert the following replacement paragraph:

As an example, the mature part of human MP121 (amino acid 237 to 352 in SEQ ID ~~NO:2~~ NO:2) with an additional 13 amino acids, including six histidines at the N-terminus, (MHHHHHHKLEFAM) (SEQ ID NO:40) was expressed in the prokaryotic vector pBP4. This vector is a pBR322 derivative having tetracyclin resistance which, in addition, contains the T7 promoter from the pBluescript II SK plasmid (Stratagene). Furthermore, the vector contains a ribosomal binding site following the T7 promoter and a start codon followed by six codons for histidine. A terminator (TØ) follows after several single restriction cleavage sites such as EcoRI, XhoI, SmaI and ApaI, for the insertion of inserts as well as stop codons in all three reading frames. In order to obtain the cDNA for the mature part of MP121, PCR was carried out on the plasmid SK121L9.1 (DSM dispositary number: 9177) using the two oligonuleotides GAATTCGCCATGGGCATCGACTGCCAAGGAGG (SEQ ID NO:41) and CCGCTCGAGAAGCTTCAACTGCACCCACAGGC (SEQ ID NO:42). Both oligonucleotides contain additional restriction cleavage sites at their ends (EcoRI and NcoI or XhoI and HindIII). In an intermediate step the resulting 377 bp fragment was cloned with blunt ends into the pBluescript II SK vector (Stragene) that had been cleaved with EcoRV. One clone in the orientation of the 5' end of MP121 towards the T7 promoter was cleaved with EcoRI and the resulting insert (0.38 kb) was cloned into the pBP4 vector that had also been cleaved with EcoRI. The correct orientation of the insert in the resulting plasmid pBP4MP121His was established by restriction analysis and sequencing. The plasmid pBR4MP121His was deposited on ~~the~~ 30.1.1995 at the DSM (depositary number: 9704). The expression of MP121 protein can be achieved by simultaneously providing T7 RNA polymerase. T7 RNA polymerase can be provided by various methods such as, e.g., by a second plasmid with a gene for T7 RNA polymerase or by infection with phages which code for T7 RNA polymerase or also by special bacterial strains which have integrated the gene for T7 RNA polymerase. The mature MP121 protein with a His-tag (MP121His) is produced in inclusion bodies by using the bacterial strain BL21 (DE3)pLysS (Novagen, #69451-1) and inducing the T7 RNA polymerase expression with IPTG according to the manufacturer's instructions. In SDS polyacrylamide gels (15%) the protein exhibits an apparent molecular weight of nearly 16 kD

(theoretical molecular weight: 14.2 kD) as is shown representatively in the Western blot of Fig. 3. The bacteria transformed with pBP4 as controls do not show any staining of specific bands. Due to the His-tag this protein can be purified on nickel-chelating agent columns as described, for example, by Hochuli et al., (Bio/Technology, Vol. 6, 1321-1325 (1988)). An additional purification is possible by means of reversed phase HPLC. A reversed phase column (Nucleosil 300-7C4 from Macherey-Nagel, Type 715023) was used with a flow-rate of 2 ml/min and an acetonitrile gradient in 0.1% TFA of 0 to 90% within 100 minutes. MP121His elutes under these conditions after ca. 40% acetonitrile.

D10

Page 26, lines 6-12, insert the following replacement paragraph:

Additionally, monoclonal antibodies were developed in mice. A peptide of 26 amino acids from the mature part of MP121 was used as an antigen: PLSLLYYDRDSNIVKTDIPDMVVEAC (SEQ ID NO:43). The antigen was coupled to ovalbumin using the free SH group of the cysteine according to conventional methods. Other constructs could be used as antigens also, as for example, the dimeric mature MP121.

D11

Page 28, lines 19-33, insert the following replacement paragraph:

PCR reactions and intermediate cloning ~~was~~ were necessary in order to shorten the 5' and 3' untranslated regions of the initial plasmid SK121L9.1 (DSM, depositary number: 9177) and to insert single restriction cleavage sites at the ends. All PCR reactions were carried out using the plasmid SK121L9.1 (DSM depositary number: 9177). In order to shorten the 5' untranslated end, the primer CCCGGATCCGCTAGCACCATGACCTCCTCATTGCTTCTG (SEQ ID NO:44) with an inserted BamHI and NheI restriction cleavage site was used in a PCR with an internal primer (CCCTGTTGTCCTCTAGAAGTG) (SEQ ID NO:45). In an intermediate step, the fragment obtained was cloned into Bluescript SK (Stratagene), sequenced and checked for concurrence with the sequence shown in SEQ ID ~~NO:1~~ NO:1. The SphI/EcoRI fragment (0.22 kb) from the plasmid pBR4MP121His was used to shorten the 3' untranslated end.

D12

Page 32, lines 7-22, insert the following replacement paragraph:

Total RNA from various tissues (brain, heart, kidney, liver, lung, spleen, muscle, ovary, testes) was isolated according to standard methods from six-week-old mice as well as from embryonic stem cells. 10 μg total RNA was used in each case in a RNAse protection assay (RPA) from Ambion (RPA II kit, #1410) according to the manufacturer's instructions. In order to obtain specific probes for activin β_A and activin β_B the genomic DNA from the mouse (129Sv) was amplified from the mature part of the proteins using corresponding specific primers. In order to facilitate cloning, EcoRI and/or BamHI or HindIII restriction cleavage sites were introduced, respectively, at the ends of the primers. In the case of activin β_A the primers were derived from mRNA from rats (GenBank Accession #M37482):

GGATCCGAATTCGGCTTGGAGTGTATGGCAAGG (SEQ ID NO:46)

and GGATCCGAATTCCTCTGGGACCTGGCAACTCTAG (SEQ ID NO:47).

Page 32, lines 23-27, insert the following replacement paragraph:

In the case of activin β_B degenerate primers were derived from the human sequence (Mason et al., Molecular Endocrinology 3, 1352-1358 (1989)):

GAGAATTCCA(GA)CA(GA)TT(TC)TT(CT)AT (SEQ ID NO:48)

and GCAAGCTTT(GA)TA(TC)TC(GA)TC(GA)TC (SEQ ID NO:49).